CHAETOMIN, A NEW ANTIBIOTIC SUBSTANCE PRODUCED BY CHAETOMIUM COCHLIODES

I. Formation and Properties[1]

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Our knowledge of the production of antibiotic substances by fungi has been largely limited to the Hyphomycetes, especially the genera *Penicillium* and *Aspergillus*. Only very few members of the other groups of fungi were found capable of inhibiting the growth of microorganisms, including bacteria, through the production of such substances. In a survey of the distribution of antagonistic fungi in the soil (Waksman and Horning, 1943), over 160 cultures were isolated. Only one of these produced a perithecium, thus establishing its position among the Ascomycetes; this organism proved to be a species of *Chaetomium*, now identified as *C. cochliodes*. When grown on suitable media, this culture produced an antibiotic substance active principally against gram-positive bacteria. This substance is designated as *chaetomin*.

FORMATION OF CHAETOMIN

C. cochliodes does not sporulate readily when grown in synthetic media. However, on media containing peptone as a source of nitrogen, such as the glucose peptone agar, abundant dark green perithecia are produced, which later turn black. Czapek-Dox medium supplemented with a small amount of corn steep proved to be most suitable for the production of chaetomin, as table 1 shows. The addition of $CaCO_3$ to the medium has an additional favorable effect upon the chaetomin formation, especially during the early stages of incubation of the cultures. This is of particular interest since the organism apparently does not produce any acid, the reaction of the medium always tending to turn alkaline. The presence of $CaCO_3$ was particularly favorable in submerged cultures, as table 2 brings out. Different carbohydrates, including brown sugar, lactose, and glucose, were tested as sources of energy for the growth of the organism and for the production of chaetomin. All were found to be about equally effective.

In order to determine the nature of the essential factor present in the corn steep that is favorable for the production of chaetomin, a number of different plant juices were tested. Only one, the fresh juice of cabbage, could compare with corn steep, especially for submerged cultures. These results, however, could not always be duplicated as was the case with corn steep; hence, the latter was always employed. A typical experiment illustrating the relation

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between the nutrition of the organism and the production of chaetomin is presented in table 3. In submerged cultures rapid growth sets in immediately, followed by a gradual accumulation of chaetomin. In stationary cultures

TABLE 1

Influence of corn steep upon the production of chaetomin

Bacillus subtilis dilution units per ml

CORN STEEP	$CaCO_3$ ADDED	7 DAYS		13 DAYS	
		S. aureus	*B. mycoides*	*S. aureus*	*B. mycoides*
%					
0	+	0	0	75	30
0	−	0	0	20	<20
0.5	+	100	75	200	30
0.5	−	30	20	75	20
1.5	+	100	30	100	50
1.5	−	30	20	100	50
3.0	+	50	20	150	30
3.0	−	0	0	150	50

TABLE 2

Influence of reaction and $CaCO_3$ upon the productuon of chaetomin in glucose media

STATIONARY CULTURES				SUBMERGED CULTURES			
Age of culture	$CaCO_3$ added	*S. aureus*	*B. mycoides*	Age of culture	$CaCO_3$ added	*S. aureus*	*B. mycoides*
days				*days*			
9	+	75	10	3	+	100	75
9	−	25	10	3	−	0	0
13	+	100	25	4	+	500	200
13	−	75	20	4	−	100	75
				6	+	1,000	750
				6	−	750	500

TABLE 3

Nutrition of Chaetomium cochliodes and production of chaetomin

30 g glucose, 5 ml corn steep, 2 g KNO_3, 0.5 g KH_2PO_4, 0.5 g $MgSO_4$ and 0.5 g KCl each; pH 7.0; $CaCO_3$ added.

INCUBATION	SHAKEN CULTURES				STATIONARY CULTURES			
	Activity*		Sugar	Total N in mycelium	Activity		Sugar	Total N in mycelium
days		*pH*	*g/L*	*mg*		*pH*	*g/L*	*mg*
3	0	7.1	26.1	40.6				
5	250	7.9	2.5	61.5	75	6.7	33.4	15.8
7	300	8.4	0.8	66.6	90	6.9	30.7	22.4
10	>1,000	8.2	0.6	68.6	750	7.0	23.4	30.0
14	2,000	8.6	0.4	63.5	225	7.0	16.8	34.7
20					200	7.2	14.9	

* *B. subtilis* dilution units.

growth is much slower, but the production of chaetomin is relatively more rapid, although it never reaches so high a point as it does in a submerged state.

TABLE 4

Production of chaetomin by different species of Chaetomium

NAME OF ORGANISM	UNITS OF ACTIVITY			
	S. aureus	*B. subtilis*	*B. mycoides*	*S. lutea*
C. globosum	0	0	0	0
C. coprinum	0	0	0	0
C. funiculum	5	10	5	10
C. elatum	5	5	10	10
C. ochraceus	0	0	0	0
C. atterinum	0	0	0	0
C. cochliodes	30	10	5	10
*C. cochliodes**	200	100	75	>300

* Our culture.

TABLE 5

Bacteriostatic spectrum of chaetomin as compared with penicillin

	CHAETOMIN*	PENICILLIN†
Bacillus subtilis 0	175,000	19,000,000
B. subtilis 970	30,000	
B. mycoides 0	40,000	5,000
B. cereus	1,500	
B. mesentericus	60,000	
B. megatherium	60,000	1,900,000
Staphylococcus aureus	500,000	9,500,000
Mycobacterium phlei	20,000	
Sarcina lutea	200,000	38,000,000
Phytomonas michiganensis	200,000	
P. prunei	200,000	
P. phaseoli	200,000	
Escherichia coli	<2,000	<5,000
Serratia marcescens	<2,000	<5,000
Aerobacter aerogenes	<2,000	<5,000
Proteus vulgaris	<2,000	<5,000
Listerella monocytogenes	20,000	300,000
Salmonella sp.	<2,000	<10,000
Pseudomonas fluorescens	1,000	<5,000
Clostridium butyricum	6,000	
Pseudomonas aeruginosa	<2,000	<5,000

* Crude preparation, on dry, organic matter basis.

† Purified preparation, testing 470 Oxford units.

A number of different species of *Chaetomium* were obtained from culture collections and tested, under uniform conditions of culture, for their capacity to produce chaetomin (table 4). Most of the cultures showed no antibacterial

action at all when grown in either a stationary or a submerged state. Even those cultures that had some activity, however, could not compare with the strain of organism used in these studies.

An attempt was next made to isolate the chaetomin from the culture medium. Various solvents were employed. Extraction with ether gave good results. The solvent was evaporated and the chaetomin dissolved in alcohol. The yield was about 30 to 75 mg of dry crude material per liter. This had an activity ranging from 80,000 to 1,000,000 *Staphylococcus aureus* and from 40,000 to 400,000 *Bacillus subtilis* dilution units.

ANTIBACTERIAL SPECTRUM OF CHAETOMIN

A comparative study was made of the antibacterial spectrum of chaetomin. The isolated crude preparation was used for this purpose. Since chaetomin resembles penicillin in its activity, a highly purified preparation of the latter was also used for comparative purposes. The antibacterial spectra for the two antibiotic substances were similar, but not identical (table 5). Both substances act principally against gram-positive bacteria and only to a limited extent upon gram-negative organisms, with considerable variation in action upon the different organisms within each group.

Chaetomin was also found to possess marked bactericidal properties (Waksman, Bugie, and Reilly, 1944). Tests for toxicity and *in vivo* activity showed that the material is not very toxic to animals. However, it gave no protection *in vivo* against various bacteria that were shown to be very sensitive to the substance *in vitro*. It still remains to be determined what mechanism or agent in the animal body inactivates or neutralizes the activity of the chaetomin.

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